RESPIRATION IN CORN WITH SPECIAL REFERENCE TO CATALASE

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RESPIRATION IN CORN WITH SPECIAL REFERENCE TO CATALASE

C. W. Lantz

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Introduction

Considerable attention has been given in recent times to the relation supposedly existing between catalase content and respiration. Catalase is an enzym almost universally present in living cells, with the power of breaking hydrogen peroxid into water and molecular oxygen.

It has long been known that plant juices and animal fluids will decompose hydrogen peroxid, liberating oxygen. Schönbein (29) considered this to be a characteristic of all "ferments." Loew (21), working with tobacco leaves, extracted a substance which had the power of liberating oxygen from hydrogen peroxid and to which he gave the name *catalase*. The power of breaking hydrogen peroxid into water and oxygen is not a characteristic of enzyms in general, but is peculiar to this specific enzym, which is usually associated with other enzyms. Loew considered catalase to be universally present in all living cells. He thought its probable function to be to destroy hydrogen peroxid and possibly other compounds of the nature of peroxids formed in respiration, which would be injurious to protoplasm if allowed to accumulate.

Appleman (1, 2), working with potatoes, found a striking correlation between catalase activity and respiration. Crocker and Harrington (13) found a close correlation between catalase activity and respiration in Johnson grass seed, but no correlation between catalase activity and respiration in the seeds of Amaranthus. Rhine (26) concluded that in germinating seeds in the early stages catalase activity at first decreases while respiration shows a gradual increase. In later stages of germination, both catalase activity and respiration increase. Burge and Neill (10, 11) found that the ingestion of foods produces an increase in catalase in the blood which parallels the increase in oxidation. Burge (8) finds that "whatever increases oxidation in the animal produces an increase in catalase . . . and whatever decreases oxidation produces a decrease in catalase by diminishing its output from the liver and by direct destruction." More recently Burge and Burge (9), working with Spirogyra, concluded that the same relationship probably exists between catalase and oxidation in plants as in animals, and they suggest that catalase may be the enzym in both plants and animals principally responsible for respiratory metabolism.

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Harvey (17) found that a blue green alga (*Phormidium laminosum*) growing in hot springs at a temperature of 65° to 73° C. contains no catalase. This fact indicates that catalase is not universally present in living cells and is not essential to respiration. Rywosch and Rywosch (28) showed that certain anaërobic bacteria contain no catalase. More recently McLeod and Gordon (22) and Callow (12), working with anaërobic bacteria, found no catalase. They consider it probable that catalase destroys some injurious substance such as hydrogen peroxid which is formed by aërobic organisms and by anaërobic organisms in the presence of oxygen. Thus anaërobic organisms lacking catalase would be seriously injured in the presence of oxygen. They offer some, but not very conclusive, evidence to support this theory.

Shaffer (30) concluded that catalase protects the tissues from excessive and injurious oxidation by destroying peroxids formed in metabolism. Catalase would thus act as a brake on excessive oxidation. Batelli and Stern (6) find that catalase prevents excessive oxidation caused by ferrous sulfate.

Morgulis (23) found that the exposure of frogs to widely different temperatures which effect changes in metabolic rate of from 300 to 400 percent had no influence on their catalase; hence he concluded that catalase is not a measure of metabolic activity. Becht (7) found the blood of animals of the same species under the same conditions to vary from 100 to 1000 percent in catalase, and hence thinks catalase has little to do with oxidation. Heinicke (18), working with apple leaves, concludes that "it is probable that the ability to decompose hydrogen peroxid is a more sensitive measure of the metabolic status of the tissue than the usual chemical analysis." Baly, Heilbron, and Hudson (5) suggest that catalase may be responsible for the reducing of nitrates to nitrites in the synthesis of amino-acids and proteins in plants, but they offer no experimental evidence for this idea.

Various kinds of plant materials have been used in previous studies of catalase activity and respiration, but in most cases little or no account has been taken of the chemical composition of the material used. It has seemed that a study of catalase activity and respiration in germinating seeds of known chemical composition and grown at different temperatures might help to clear up some of the disputed points.

The work upon which this paper is based was done in the laboratory of plant physiology of the University of Illinois under the direction of Prof. C. F. Hottes. The writer gratefully acknowledges his assistance in the selection of the problem and in its development and presentation.

Materials and Methods

Materials

In 1896, Professor Hopkins (19) of the University of Illinois Agricultural Experiment Station began selecting corn for protein and oil content. The

corn with which he began this work was a white corn known locally as "Burr's White." The selection has been continued to the present time, and, as a result, four strains of corn differing widely in protein and oil content have been secured. These strains are designated as "Illinois High Protein," "Illinois Low Protein," "Illinois High Oil," and "Illinois Low Oil."

Table 3 gives the analyses for protein and oil furnished by the Illinois Experiment Station of some of these strains, crop of 1923, used in the present experiments.

Germination

The corn was germinated on sterilized clay blocks placed in a germinating pan furnished with water kept at a constant level. The germination was carried on in constant-temperature cases maintained at 10°, 20°, and 30° C. These cases are a part of the regular laboratory equipment. The corn was first soaked for one hour in a 0.25 percent solution of "Uspulun" to prevent the growth of molds. Studies of the effect of "Uspulun" upon the germinating corn were made and the results are given later.

Determination of Catalase

The apparatus consisted of a bottle fitted with a delivery tube and a burette, so arranged that the peroxid could be introduced upon the material to be tested without changing the volume of the gas in the apparatus. The liberated oxygen was collected over water in a eudiometer tube. All gas volumes were reduced to standard conditions. The apparatus was shaken at a constant rate by a shaker run by an electric motor.

Five kernels of corn selected from different parts of the ear were used in each test. The ungerminated corn was ground in the air-dry condition in a grinder and then by means of a mortar and pestle so that it would pass through an 80-mesh sieve. The degree of pulverization is known to be important in catalase-determination. Crocker and Harrington (13) found that Johnson grass seed gave maximum results for catalase when ground to the fineness of 70- to 80-mesh sieves.

The germinated corn was rapidly dried *in vacuo* over sulfuric acid at room temperature. It was then ground and put through an 80-mesh sieve. By drying the seedlings, their preparation for the quantitative determination of catalase was made quite simple, and, as the results given later show, the drying did not affect the catalase content.

After the material was prepared, 0.15 g. was placed in the bottle with an amount of distilled water to give a volume of 15 cc. when the hydrogen peroxid was added. Owing to the limitations in the size of the apparatus, it was necessary to use only 0.075 g. of the material when the catalase content was high. In the data, these results are doubled for comparison. The determinations were carried on at room temperature, which varied from

18° to 20° C. Readings were taken at 2-, 5-, and 10-minute intervals. Only the readings at the 10-minute interval are given in the tables. All tests were made in duplicate, and the averages of these tests are given in the tables.

The peroxid used was the "Dioxygen" of the Oakland Chemical Company. This brand gave very uniform results. Dioxygen, according to the work of Crocker and Harrington (13) yields at 25° C. 13 cc. of oxygen per cc. of peroxid. The Dioxygen, which is acid, was made slightly alkaline to phenolphthalein by adding a few drops of 5 percent KOH. This is important, as acidity inhibits catalase activity. Since the peroxid deteriorates rapidly when neutral, only enough was prepared at a time for the immediate determinations.

In these determinations the amount of catalase present varied greatly. Some samples of germinated corn had fifty times the amount found in the same weight of ungerminated corn. Table 1 shows the amount of peroxid used when the catalase liberated a certain amount of oxygen in 15 minutes. A trial test was run when necessary to determine the amount of peroxid to be used for a given sample.

TABLE 1. *Amounts of "Dioxygen" Used*

Oxygen Liberated in 15 Minutes	Dioxygen Used	Oxygen Liberated in 15 Minutes	Dioxygen Used
cc.	cc.	cc.	cc.
Below 20	2.0	66–85	10.0
21–25	2.5	106–125	12.0
26–35	3.0	126–145	14.0
36–45	4.0	146–165	16.0
46–65	6.0		

Measurement of Respiration

In measuring the respiration of the corn, 15 kernels selected from different parts of the ear were taken. These were soaked for one hour in a 0.25 percent solution of "Uspulun" and then germinated in closed respiratory chambers on sterilized moist filter paper. During the respiration period, the respiring material was kept at a constant temperature in the temperature cases. The temperatures used were 10°, 20°, and 30° C. Light was excluded from the chambers to prevent photosynthesis.

The amount of carbon dioxid in the chambers was determined by means of a katharometer made by the Cambridge and Paul Instrument Company of America. The gas was removed from the chambers by means of mercury and two leveling bulbs. It was then forced by means of the mercury into the katharometer. All the gas was removed from a chamber, and it was thoroughly mixed in the bulbs and the katharometer before the test was made. This gave an accurate sample for testing.

The katharometer gives the percentage of carbon dioxid in the sample of gas. Knowing the volume of the gas in the respiration chamber and the air-dry weight of the corn used, the quantity of carbon dioxid per gram air-dry weight per hour was calculated.

Kidd (20) showed that 10 percent carbon dioxid slightly retards the respiration of white mustard seeds. In the present studies the percentage of carbon dioxid was kept below 10 percent, so that there would be little if any retardation.

After removing the gas from the chamber, the same seedlings were used for the next determination. By using several chambers it was possible to carry on simultaneously several sets of seedlings. The method here described proved to be a rapid and accurate one for measuring the rate of respiration. The whole process of taking the sample and testing it could be accomplished in 15 minutes.

Results and Discussion of Catalase Activity

The Effect of the Quantity of Peroxid Used in Catalase-determination

Morgulis (23), Rhine (26), and Baas-Becking and Hampton (3) point out that the action of catalase is a type of balanced reaction, and thus the ratio between the amount of catalase and the amount of peroxid used is important. Morgulis shows that an excess of peroxid retards catalase activity. Hence the use of large quantities of peroxid to determine small quantities of catalase is objectionable. He states that within the limits of 60 to 100 percent decomposition of the peroxid, the amount of oxygen liberated is practically a linear function of the quantity of catalase. For liver catalase, the maximum oxygen evolution occurs when 65 to 70 percent of the total peroxid is decomposed.

In order to determine the effect of the quantity of peroxid used upon the catalase activity, tests were made upon equal quantities of the same material, all conditions being made constant except the quantity of peroxid used. In a period of 15 minutes 41 cc. of oxygen were liberated when using 10 cc. of H_2O_2, and 57.5 cc. of oxygen when 5 cc. of H_2O_2 were used. Several similar tests were made, all of which gave the same results, showing that an excess of peroxid retards catalase activity. In comparative studies it is very necessary to take due precaution against errors due to the use of large amounts of peroxid. In all the measurements of catalase activity in the following experiments the amount of peroxid used was such that from 60 to 90 percent of the peroxid was decomposed in 15 minutes. The amount to be used in any test was determined by a preliminary trial.

Effect of Drying the Seedlings on Catalase Content

The simplest method of preparing the corn seedlings for a quantitative determination of catalase was to dry them before grinding. This not

only made easy the preparation of the material, but removed the necessity of a correction for moisture. However, before this method was adopted, tests were made to see what effect the drying would have upon the catalase activity. Crocker and Harrington (13) found that the drying of peach seeds cut in pieces decreased the catalase activity. Rose (27) found that drying after-ripened Tilia seeds for two days at room temperature had no effect on the catalase.

In the first trials, the seedlings were dried in a current of hot air at a temperature of 56° C. The material was then prepared and tested in the usual way. The results secured in this way were compared with the results obtained by testing seedlings of the same age grown from kernels of the same ear which were not dried before testing. Corrections were made for moisture so that the amount of dry material tested would be the same.

High Oil corn germinated at 20° C. for 2 days and tested for catalase without drying, corrections being made in the weight for moisture, gave 22.1 cc. of oxygen in 10 minutes, while corn from the same ear germinated for the same length of time under similar conditions, but dried rapidly at a temperature of 56° C. before grinding, gave only 14 cc. of oxygen in 10 minutes. Low Oil corn seedlings tested without drying gave 8.2 cc. of oxygen in 10 minutes, while similar seedlings dried at 56° C. gave 6.6 cc. of oxygen in 10 minutes. These facts show that drying the seedlings at a temperature of 56° C. decreased the catalase content of the seedlings.

Tests were then made to see if drying *in vacuo* over sulfuric acid would affect the catalase. Low Oil corn germinated for 4 days at 20° C. and tested without drying, corrections being made for moisture, gave 64.5 cc. of oxygen in 10 minutes, while similar seedlings dried rapidly *in vacuo* over sulfuric acid gave 64.7 cc. of oxygen in 10 minutes. High Oil corn germinated for 3 days at 20° C. and tested without drying gave 90 cc. of oxygen in 10 minutes, and similar seedlings dried *in vacuo* over sulfuric acid gave 86 cc. of oxygen in 10 minutes. These results indicate that drying the seedlings *in vacuo* over sulfuric acid had little effect on the catalase content. The difference in catalase content of the two lots is even less than one might expect, considering the individual differences in the lots and the difficulty in correcting for the moisture in the material that was not dried.

Effect of "Uspulun" upon Catalase Activity

It has already been stated that the corn was treated with "Uspulun" in order to prevent the growth of molds. "Uspulun" is a chlorophenol-mercury compound made by the Bayer Chemical Company, Inc., New York. The treatment consisted in soaking the corn for one hour in a 0.25 percent solution of Uspulun. The corn was then placed without rinsing in water directly on the sterilized clay blocks or in the respiration chambers. This treatment effectively prevented the growth of molds and, as will appear later, did not in the least affect the catalase or the respiration.

A number of statements can be found claiming stimulating properties for Uspulun. Czarnowski (14), however, found that the rootlets and plumules of wheat grains sterilized with a 0.59 percent solution of Uspulun were smaller and weighed less than those of similar grains soaked for the same length of time in water.

Before using Uspulun as a fungicide, the following experiments were made to find what would be its effect upon the germination and catalase activity. The corn was divided into 3 lots. One was soaked for one hour in a 0.25 percent solution of Uspulun, the second for one hour in water, and the third was placed directly on the germinator block without treatment. Table 2 shows the effect of these treatments upon germination and catalase activity.

TABLE 2. *Effect upon Germination and Catalase Activity of Soaking Corn in Water and in "Uspulun"*

Kind of Corn	Germination	Treatment	Percentage Increase in Weight	Catalase Content (cc. O_2 in 10 Min.)
High Protein no. 20	3 days at 20° C...	None.....	43.8	75.2
		One hour in water...	51.7	85.9
		One hour in Uspulun.	45.5	88.7
Low Protein no. 15	3 days at 20° C...	None.....	43.6	53.9
		One hour in water...	54.0	61.0
		One hour in Uspulun.	64.0	60.3
High Oil no. 15...	3 days at 30° C...	None.....	119.7	137.4
		One hour in water...	130.8	173.0
		One hour in Uspulun.	127.7	166.3
Low Oil no. 4.....	3 days at 30° C...	None.....	49.8	68.9
		One hour in water...	64.1	52.3
		One hour in Uspulun.	59.0	58.6

A study of these results shows that both soaking in water and soaking in Uspulun increased the growth and the catalase activity. Low Oil corn germinated for 3 days at 30° C. seemed to furnish an exception to this rule. However, the Low Oil corn was always quite irregular in its germination and growth, and this apparent exception is of no special significance.

In general, it can be said that soaking in Uspulun had no stimulating effect upon the growth or catalase activity above what is secured by soaking for the same period in water. As a fungicide, it effectively prevented the growth of molds without injury to the corn.

Catalase in Ungerminated Corn

Table 3 shows the catalase content in the ears of the different strains of corn. The averages indicate the highest catalase content for the High Protein and High Oil strains. Of the other two strains, the Low Oil is quite consistently the lower. Within any one of the given strains, there exists no constant and close correlation between the percentage of protein

or oil and the catalase content. In the High Protein and High Oil strains, both of which average higher in catalase than the other strains, occasional ears are found whose catalase content is as low as that found in the Low Protein and Low Oil strains.

TABLE 3. *Summary of Catalase in Corn of Different Composition*

High Protein		High Oil		Low Protein		Low Oil	
% Protein	Volume of Oxygen in 10 Min.	% Oil	Volume of Oxygen in 10 Min.	% Protein	Volume of Oxygen in 10 Min.	% Oil	Volume of Oxygen in 10 Min.
	cc.		cc.		cc.		cc.
14.01	10.5	8.68	7.3	6.51	9.1	1.40	6.4
16.45	11.2	9.47	8.5	7.61	7.2	1.49	6.3
17.68	16.6	9.57	10.0	7.61	7.0	1.64	5.0
17.90	8.0	10.06	14.2	7.70	6.7	1.64	6.3
18.13	20.0	10.08	10.2	8.02	6.5	1.85	9.9
18.34	14.9	10.29	19.4	8.71	7.4	1.93	5.8
Average	13.5		11.6		7.3		6.6

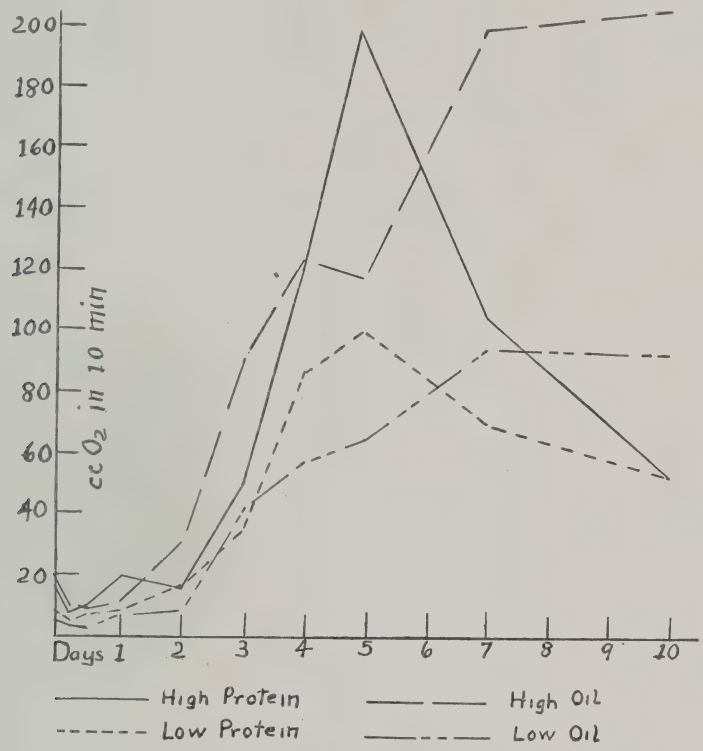
Catalase in Germinating Corn

It has been noted by different investigators that there is a rapid rise of catalase during germination. Deleano (15) found an increase in catalase during the germination of castor beans which continued until the ninth day, after which time there was a decrease. He noted that the catalase decreased as the fat decreased. Crocker and Harrington (13) found a large increase of catalase during the germination of Sudan grass and Johnson grass seeds. Rhine (26) concluded that the catalase decreases slightly during the first hours of germination, but that this decrease is followed by a large and steady increase.

TABLE 4. *Catalase Activity in Germinating Corn at 10° C. (Catalase Activity Expressed in cc. of Oxygen Liberated in 10 Minutes)*

Germination Period	High Protein No. 2 (% Protein 16.45)		Low Protein No. 18 (% Protein 7.61)		High Oil No. 20 (% Oil 9.57)		Low Oil No. 14 (% Oil 1.4)	
	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase
	cc.		cc.		cc.		cc.	
None	11.2	0	7.2	0	10.0	0	6.4	0
1 day	13.9	14.2	8.5	8.2	9.9	20.8	6.4	22.3
2 days	6.0	17.7	6.6	20.3	6.0	29.5	4.5	24.6
3 days	11.6	23.5	5.6	27.3	5.3	34.7	2.2	34.9
4 days	13.2	27.8	11.8	32.7				
5 days	30.2	36.8	17.4	34.3	10.1	45.3	1.4	44.5
7 days	60.6	44.5	24.5	36.9	34.7	50.5	6.2	48.9
10 days	89.4	59.8	49.2	43.4	52.8	59.7	21.2	48.4
12 days	87.8	76.0	52.2	62.7				
15 days	88.5	64.6	64.2	65.2	99.9	75.9	41.7	55.4
20 days	131.0	66.5	68.4	72.9	150.9	98.1	51.5	67.3
30 days	131.7	105.1	57.7	89.8				

Table 4 shows the catalase activity of seedlings of the different strains of corn germinated at 10° C. The germination was very slow at 10° C. The radicles appeared the 6th day in the High Protein corn, and on the 7th day in the Low Protein and High Oil corns, while in the Low Oil corn they did not appear until the 10th day. A study of these data shows that there is a gradual rise in catalase activity which begins just before the appearance of the radicle. It can also be noted that in the early stages of germination there is, in all four strains of corn, a decrease in catalase below the quantity found in the ungerminated corn. The lowest point is



TEXT FIG. 1. Catalase activity in germinating corn kernels of different composition at 20° C. High Protein no. 37; Low Protein no. 97; High Oil no. 15; Low Oil no. 14.

on the 2d day of germination in the "High Protein" corn, on the 3d day in the Low Protein and High Oil corns, and on the 5th day in the Low Oil corn. These results are in accordance with the fact that at this temperature the High Protein corn showed the most rapid germination and the Low Oil corn the slowest. After germination was well started, the catalase activity of the Low Protein and Low Oil corns was always below that of the other two strains. The Low Oil was always the lowest.

Tables 5 and 6 and text figure 1 show the catalase activity during

TABLE 5. *Catalase Activity in Germinating Corn at 20° C. (Catalase Activity Expressed in cc. of Oxygen Liberated in 10 Minutes)*

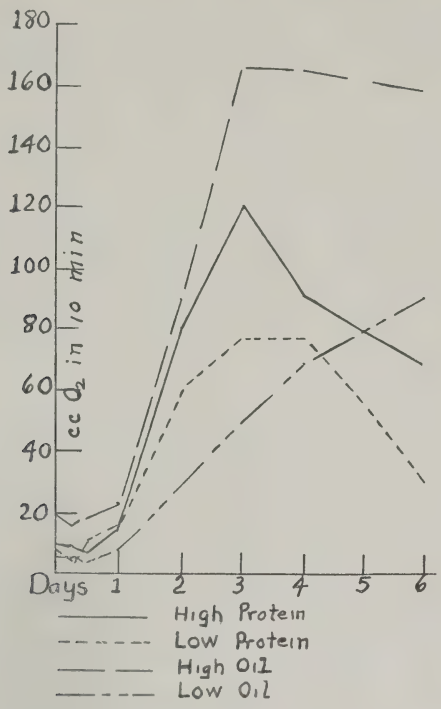
Germination Period	High Protein No. 37 (% Protein 17.68)		Low Protein No. 99 (% Protein 6.51)		High Oil No. 15 (% Oil 10.29)		Low Oil No. 14 (% Oil 1.4)	
	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase
	cc.		cc.		cc.		cc.	
None	16.6	0	9.1	0	19.4	0	6.4	0
6 hours	7.8	14.5	6.5	19.8	11.4	15.3	4.6	12.7
12 hours	10.5	20.5	8.5	26.5	9.7	21.3	4.1	17.4
1 day	19.7	20.1	8.1	23.0	11.0	31.2	7.6	17.1
2 days	15.9	28.4	16.9	33.1	31.5	43.5	8.2	26.1
3 days	49.8	33.6	34.8	39.8	85.5	73.4	40.2	
4 days	119.3	48.9	85.5	57.3	122.0	87.0	58.9	39.2
5 days	197.9	86.2	99.7	147.0	117.1	131.7	64.5	50.5
7 days	102.8	264.3	74.3	148.0	197.8	265.0	93.0	116.8
10 days	52.6	545.8	52.7	338.6	203.3	367.9	92.4	254.5

TABLE 6. *Catalase Activity in Germinating Corn at 20° C. (Catalase Activity Expressed in cc. of Oxygen Liberated in 10 Minutes)*

Germination Period	High Protein No. 2 (% Protein 16.45)		Low Protein No. 18 (% Protein 7.61)		High Oil No. 20 (% Oil 9.57)	
	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase
	cc.		cc.		cc.	
None	11.2	0.0	7.2	0.0	10.0	0.0
6 hours	7.5	13.3	5.2	14.2	8.0	18.2
12 hours	7.4	20.7	5.8	20.5	9.5	28.1
1 day	14.9	26.0	7.5	24.4	11.4	32.0
2 days	11.9	27.1	11.9	29.3	22.1	39.0
3 days	62.6	37.3	32.8	37.4	78.3	
4 days	98.7	48.4	38.3	49.5	132.5	59.2
5 days	100.3	77.0	70.7	66.5	201.9	104.5
7 days	117.3		84.3	102.8	242.2	195.1
10 days	103.5	249.3	81.3		206.2	352.5

germination at 20° C. There is a rapid rise in catalase beginning just before the appearance of the radicle. This rise occurred about 24 hours later in the Low Oil corn than in the other strains, since the Low Oil corn was always slower in its germination at 20° C. There is also a drop in the catalase content below that found in the ungerminated corn. This occurred during the first 12 hours of germination in the Low Oil corn and during the first 6 hours in the other strains. The period of increase in catalase during germination was followed by a decrease if the seedlings were allowed to grow and exhaust the stored foods. For example, the kernels of High Protein corn no. 37 were unusually small and the seedlings grew quite rapidly, thus quickly exhausting the stored foods, and as a result the catalase dropped sooner and more rapidly than in the other corns. The kernels of Low Oil corn no. 14 were large and germination was slow, consequently the catalase had not begun to decrease at the end of 10 days of

germination. This decrease in catalase during germination and growth following an increase is in agreement with the results of the work of Deleano (15).



TEXT FIG. 2. Catalase activity in germinating corn kernels of different composition at 30° C. High Protein no. 37; Low Protein no. 97; High Oil no. 15; Low Oil no. 14.

Tables 7 and 8 and text figure 2 show catalase activity in corn germinated at 30° C. Since germination is much more rapid at 30° C. than at 10°

TABLE 7. *Catalase Activity in Germinating Corn at 30° C. (Catalase Activity Expressed in cc. of Oxygen Liberated in 10 Minutes)*

Germination Period	High Protein No. 37 (% Protein 17.68)		Low Protein No. 97 (% Protein 6.51)		High Oil No. 15 (% Oil 10.29)		Low Oil No. 4 (% Oil 1.64)	
	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase
	cc.		cc.		cc.		cc.	
None.....	9.8	0	7.7	0	19.4	0	5.1	0
6 hours....	8.1	16.7	3.7	21.7	15.1	19.3	5.7	17.7
12 hours....	6.8	25.6	10.0	27.7	18.6	26.0	3.2	19.7
1 day.....	14.2	33.1	16.2	37.8	23.5	35.2	7.1	34.8
2 days.....	81.2	47.6	60.9	58.3	90.3	55.9	25.2	42.0
3 days.....	120.2	112.6	77.7	86.2	166.0	127.7	49.6	64.1
4 days.....	95.7	203.6	77.9	144.0	164.2	154.4	69.3	121.1
5 days.....	79.5		56.8	302.7				
6 days.....	70.3	463.7	29.5	542.0	158.0	369.4	91.3	219.9

TABLE 8. *Catalase Activity in Germinating Corn at 30° C. (Catalase Activity Expressed in cc. of Oxygen Liberated in 10 Minutes)*

Germination Period	High Protein No. 2 (% Protein 16.45)		Low Protein No. 18 (% Protein 7.61)		High Oil No. 20 (% Oil 9.57)		Low Oil No. 14 (% Oil 1.40)	
	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase	Catalase	% Weight Increase
	cc.		cc.		cc.		cc.	
None	11.2	0.0	7.2	0.0	10.1	0.0	6.4	0.0
6 hours	11.2	13.7	6.9	17.4	9.0	22.9	2.8	21.4
12 hours	10.5	23.3	8.6	31.1	8.9	30.0	5.2	28.6
1 day	10.5	31.7	12.4	32.1	16.1	43.5	6.2	36.6
2 days	60.0	47.6	63.0	52.9	57.0	77.0	25.9	48.1
3 days	78.9	109.5	53.1	81.5	88.0	128.6	76.2	74.8
4 days	131.9	240.7	87.2	175.4	136.7	221.7	72.1	147.9
5 days	118.5	288.8	72.0	234.7	159.0	560.8	91.4	
6 days	79.9	312.0	52.1	350.8				
7 days	31.3	415.7	22.1	500.6	91.8	697.7	44.7	292.4

or 20° C., the rise in catalase came earlier, starting before the end of 24 hours of germination. A drop in the catalase occurred before this rise, as in the germination at the other temperatures. The more rapid exhaustion of the stored food in the kernels due to more rapid growth was shown by an earlier drop in catalase. By comparing the catalase content of the seedlings at this temperature with the content at 20° C., it is apparent that the seedlings grown at 30° C. show regularly less catalase at its optimum than the seedlings grown at 20° C. One exception was found in High Protein corn no. 2, which showed a little more catalase at 30° C. than at 20° C., but in no case did the higher temperature cause any marked increase in catalase. In the High Oil strains, the higher temperature checked catalase activity. The curves showing the catalase activity of the different strains of corn during germination at 30° C. parallel each other more closely than those showing the catalase of seedlings at 20° C.

TABLE 9. *The Effect of a Temperature of 42° C. upon the Catalase Activity of High and Low Protein Corn (Corn Germinated at 30° C. for 3 Days, then the Temperature Was Increased to 42° C. for 2 Days and then Restored to 30° C.)*

Kind of Corn	Germination Period							
	Temp., 30° C.				Temp., 42° C.		Temp., 30° C.	
	None	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
High Protein no. 2	11.2	12.0	44.2	97.4	64.2	51.3	77.5	97.6
Low Protein no. 18	7.2	11.3	45.5	61.1	49.5	29.7	4.3	65.4

Table 9 shows the effect of a still higher temperature on catalase. The seedlings of the High Protein and Low Protein corns which were

germinated at 30° C. were subjected for 48 hours to a temperature of 42° C. at the beginning of the 4th day. The temperature was restored to 30° C. at the beginning of the 6th day. The seedlings were injured at the temperature of 42° C. and the catalase dropped, going quite low in the Low Protein seedlings. After the temperature was restored to 30° C., the seedlings recovered, growth began, and the catalase increased. The recovery took place more rapidly in the High Protein corn.

A comparison of the results obtained at the different temperatures shows some interesting facts concerning catalase in germinating corn. There was, at all three temperatures and in all four strains of corn, a drop in catalase at the beginning of germination. This was followed by a rapid increase in catalase activity during the growth of the seedlings. This increase was followed by a decrease which was associated with the disappearance of the stored food in the endosperm. The drop in catalase at the beginning of germination was noted by Rhine (26). The facts that this drop occurred at all temperatures, and that the subsequent rise came later in the slowly growing seedlings at 20° C., indicate that it is one of the regular physiological occurrences during the germination of corn. Since metabolism increases from the beginning, catalase activity and metabolism do not parallel each other in the early stages of germination in corn.

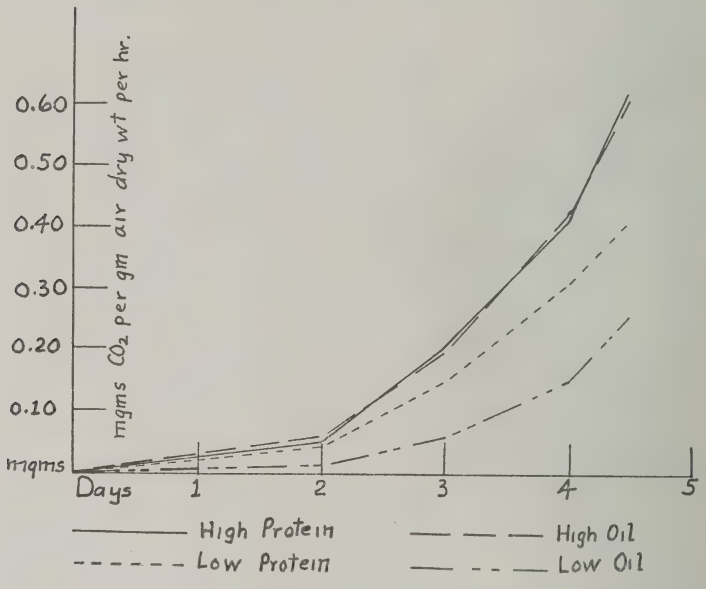
At all temperatures the seedlings of the High Oil corn showed the highest catalase content, and those of High Protein corn the next highest. At 10° and 20° C. the Low Oil corn showed the lowest catalase, but at 30° C. its catalase ultimately went as high as that of the Low Protein corn. The highest catalase activity was found during the germination of the corns at 20° C. There were only two cases in which the seedlings showed a slightly higher catalase content at 30°. The higher temperature seemed to check catalase activity although growth was much more rapid. At 42° C. the catalase almost disappeared in the Low Protein corn. At 10° C. there was a gradual accumulation of catalase, so that at the end of 30 days the amount of catalase in the High Protein corn no. 2 was as high as at the other temperatures. This was the case although growth was very slow, and although the stage of germination at the end of 30 days was much less advanced than at the 7th day of germination at 20° C. It appears, then, that the catalase content of corn seedlings germinated at 10° C. is higher and the catalase content of corn seedlings germinated at 30° C. is lower than one would expect, if catalase activity is a measure of metabolism.

Results and Discussion of Respiration

Respiration in air-dry seeds is low. White (32) found that corn stored in air-dry condition gave off 0.004 mg. of carbon dioxid per g. air-dry weight in 24 hours, while some other kinds of seeds gave off no appreciable amount. With the imbibition of water, respiration increases, becoming quite marked according to Bailey (4) when the moisture content of the corn

reaches 13 percent. As germination proceeds, respiration increases very rapidly.

Temperature, too, is responsible for a rapid increase in respiration. Palladin says that "the rate of gaseous exchange is nearly proportional to the temperature, for the medium temperatures, but a maximum rate is reached at about 40° C. and a further rise in temperature is without influence upon this rate which remains constant until death intervenes." Detmer (16) and Ziegenbein (33) studied the respiration of seedlings in relation to temperature. They found an increase in respiration with an increase in temperature up to 40° C. They located an optimum temperature at about 40° to 42° C., but it is questionable whether there is a true optimum for respiration, since many plants show an increase in respiration up to the death point.



TEXT FIG. 3. Respiration in germinating corn kernels at 20° C. High Protein no. 37; Low Protein no. 97; High Oil no. 15; Low Oil no. 4.

At 10° C., as shown in table 10, respiration was at a very low rate. At all periods for which the respiration was determined, the relative rates of respiration in the different strains of corn were as follows: High Oil corn, highest; High Protein corn, second; Low Protein corn, third; and Low Oil corn, lowest. The higher rate of respiration and growth of the High Oil corn at this temperature seems to indicate that the oil, stored largely in the embryo, becomes available readily at this temperature.

The relative rates of respiration at 20° C. in the different strains of corn are shown in table 11 and text figure 3. The High Oil corn germinated a

TABLE 10. *Summary of Respiration at 10° C. in Different Strains of Corn (CO_2 Expressed in Mg. per Hour per G. Air-dry Weight)*

Period of Germination	Series	High Protein Ear No. 2	Low Protein Ear No. 18	High Oil Ear No. 20	Low Oil Ear No. 14
First 4 days of germination.....	*A*.....	.00097	.00078	.00184	.00065
	B.....	.00079	.00059	.00179	.00066
	Average	.00088	.00068	.00181	.00065
5th to 10th day of germination .	*A*.....	.0080	.0072	.0109	.0044
	B.....	.0066	.0049	.0118	.0034
	Average	.0073	.0060	.0113	.0039
10th to 15th day of germination	*A*.....	.0177	.0123	.0201	.0109
	B.....	.0111	.0068	.0178	.0095
	Average	.0144	.0095	.0189	.0102

little faster than the others. Consequently, for the first 48 hours this corn showed the highest respiration. High Protein corn no. 37 and High Oil corn no. 15 paralleled each other quite closely in their respiration during germination and growth. Of the other two, the Low Oil corn showed the lowest rate of respiration, in accordance with the slower growth of this strain.

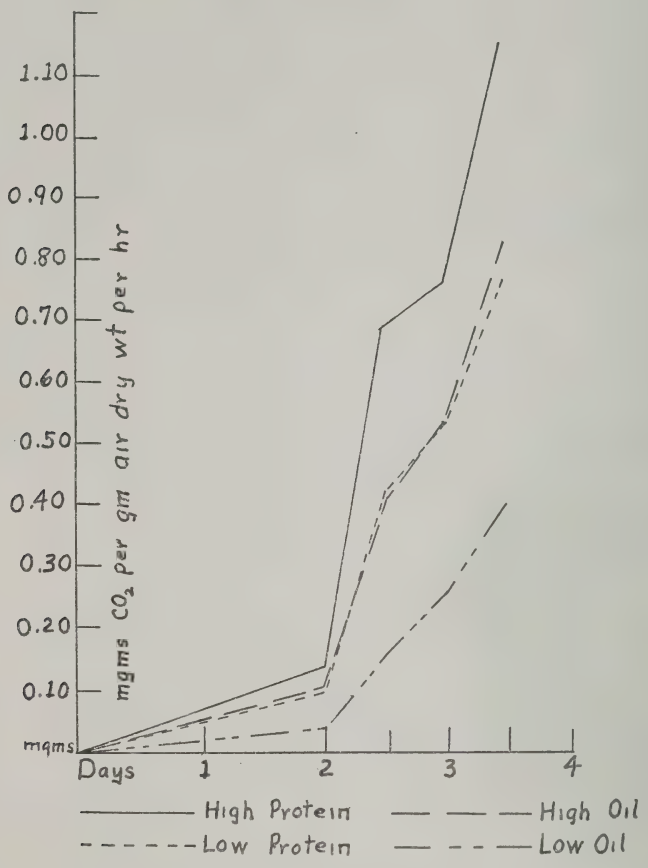
TABLE 11. *Summary of Respiration at 20° C. in Different Strains of Corn (CO_2 Expressed in Mg. per Hour per G. Air-dry Weight)*

Period of Germination	Series	High Protein Ear No. 37	Low Protein Ear No. 97	High Oil Ear No. 15	Low Oil Ear No. 4
First 48 hours of germination...	*A*.....	0.0471	0.0498	0.0583	0.0237
	B.....	0.0552	0.0481	0.0613	0.0258
	Average	0.0511	0.0489	0.0598	0.0247
Third day of germination.....	*A*.....	0.1899	0.1525	0.1884	0.0748
	B.....	0.2207	0.1520	0.1977	0.0650
	Average	0.2053	0.1522	0.1931	0.0699
Second 12 hours of the 4th day of germination.....	*A*.....	0.3322	0.3087	0.4191	0.1870
	B.....	0.5035	0.3278	0.4211	0.1154
	Average	0.4178	0.3182	0.4201	0.1512
First 12 hours of the 5th day of germination.....	*A*.....	0.5293	0.4180	0.5706	0.2679
	B.....	0.7273	*	0.6444	0.2652
	Average	0.6283	0.4180	0.6070	0.2655

* No reading.

A comparison of the respiration of lots *A* and *B* of High Protein corn germinated at 20° C. shows that lot *A* was consistently lower than lot *B* (table 11). A study of the germination of the two lots showed that, while all kernels had germinated, those of lot *A* averaged less growth than those of lot *B*. The average length of the radicles for the 15 seedlings of lot *A* at the end of the 4th day was 5 cm.; for those of lot *B*, 6 cm. These

figures show a difficulty in the making of comparisons, since the stage of germination is important.



TEXT FIG. 4. Respiration in germinating corn kernels at 30° C. High Protein no. 37; Low Protein no. 97; High Oil no. 15; Low Oil no. 4.

At 30° C. (table 12 and text figure 4), the rate of respiration was much more rapid, being two to three times the rate at 20° C. at similar periods of germination. High Protein corn no. 37 showed the highest rate of respiration, while High Oil corn no. 15 respired at about the same rate as Low Protein corn no. 97. These facts indicate that the higher temperature did not increase the rate of respiration in the High Oil corn as much as in the other strains.

A study of text figure 4 shows that the respiration in all strains of corn was more rapid for the first 12-hour period of the 3d day than for the second 12-hour period. The cause of this difference was a temperature increase. During the first 12-hour period the laboratory temperature was higher

than that desired in the case, consequently regulation was impossible. The next 12-hour period was the night period, and the temperature was regularly at 30° C. These facts show the effect of a slight rise in temperature upon respiration, and also that the optimum temperature for respiration is above 30° C.

TABLE 12. *Summary of Respiration at 30° C. in Different Strains of Corn (CO_2 Expressed in Mg. per Hour per G. Air-dry Weight)*

Period of Germination	Series	High Protein Ear No. 37	Low Protein Ear No. 97	High Oil Ear No. 15	Low Oil Ear No. 4
First 48 hours	*A*	0.1352	0.0953	0.1139	0.0537
	B	0.1400	0.1038	0.0918	0.0315
	Average	0.1376	0.0995	0.1028	0.0426
First 12* hours of 3d day	*A*	0.6310	0.4248	0.4309	0.1869
	B	0.7353	0.4089	0.3875	0.1395
	Average	0.6831	0.4168	0.4092	0.1632
Second 12 hours of 3d day	*A*	0.7122	0.4691	0.5142	0.2367
	B	0.8177	0.5929	0.5512	0.2825
	Average	0.7649	0.5310	0.5327	0.2596
Six hours on 4th day	*A*	0.9880	0.7471	0.7741	0.4339
	B	1.3206	0.7787	0.8828	0.3532
	Average	1.1540	0.7629	0.8284	0.3935

* Temperature above 30° C.

The High Protein corn at all temperatures gave a high rate of respiration. This seems to indicate that proteins may stimulate respiration. This agrees with the conclusion of Spoehr and McGee (31) that amino-acids stimulate respiration.

The respiration rate in all strains at all temperatures used was low in the early stages of germination, but it increased quite rapidly in the later stages. There was a rapid rise in respiration beginning about the second day of germination at both 20° and 30° C. This accompanied a period of rapid growth. The High Protein corn at all temperatures showed a high rate of respiration and the Low Oil corn a low rate. The highest rate at 10° C. was in the High Oil corn; at 20° C. the High Oil and High Protein strains were about equal in rate; and at 30° C. the rate of the High Protein corn was highest.

Relation of Catalase Activity to Respiration

It has already been shown in the review of literature that there is much disagreement among investigators as to the relation of catalase to respiration. Some investigators have found a close correlation between catalase and respiration, and others have found no correlation. In most cases, catalase has been related in some way to the respiratory processes.

The present investigation has given measurements of catalase activity

and of respiration in strains of corn of different chemical composition during germination at different temperatures, thus providing an excellent opportunity for comparisons of these two processes.

A comparison of the results of these determinations fails to show a close correlation between catalase activity and respiration. In the first place, a slight drop in catalase occurred in all strains at all temperatures in the early stages of germination, while respiration increased steadily from the beginning. For example, in High Protein corn no. 37 at 20° C. the catalase content was slightly lower in the germinating corn at the end of 2 days of germination than in the ungerminated corn, while the respiration of the germinating corn during this period showed an evident increase. The same condition can be noted in the strains germinated at 10° C. At the end of 4 days of germination, there was no increase in catalase, but there was a measurable respiration for this period even at this low temperature. This drop in catalase in the early stages of germination is found in all the strains at all temperatures used, and does not parallel the respiration.

A study of the catalase activity and the respiration of the different strains of corn at a given temperature gives further opportunity for comparison. At 10° C. (table 10), the highest respiratory rate during the first two weeks of germination was found in the High Oil corn, but the High Protein corn (table 4) showed the highest catalase activity for this period. The Low Oil corn showed the lowest catalase activity and the lowest respiration rate, which would seem to indicate a correlation.

At 20° C. (text figs. 1, 3), with the exception of the divergence of catalase and respiration in the early stages of germination, the catalase activity and the respiration in the different strains paralleled each other quite closely. The High Protein corn no. 37 and the High Oil corn no. 15 showed a very close correlation.

At 30° C. (text figs. 2, 4), the catalase activity and the respiration are not closely correlated in the different strains. High Oil corn no. 15 gave more catalase than High Protein corn no. 37, but the seedlings of High Protein corn respired more rapidly than those of High Oil corn. Low Oil corn no. 4 was low in both catalase and respiration in the earlier stages of germination at 30° C. At the end of 4 days of germination, however, its catalase was about equal to that of Low Protein corn no. 97, but the respiration rate of this same strain at the same period was considerably below that of the Low Protein corn. It seems, therefore, that there is little or no correlation between catalase activity and respiration at 30° C. in germinating corn.

A further study may be made by comparing the catalase content and the respiration of the seedlings as affected by different temperatures. Respiration, as shown, was much higher at 30° than at 20° C. At 10° C. the respiratory rate was very low. A study of the catalase content of the seedlings at the different temperatures shows that the optimum temperature

for catalase activity was at 20° C. in nearly every case. In the two cases in which the catalase content of a strain was higher at 30° than at 20° C., the increase was but slight and was in no way proportional to the increase in respiration. The measurement of catalase at 30° was carried on at various stages of growth of the seedlings until a drop occurred, so it can not be said that the optimum for 30° had not been reached.

It is evident that, while respiration in corn seedlings increases with an increase in temperature, there is no corresponding increase in catalase activity. In fact, the higher temperatures retard catalase activity. At 42° C., catalase activity in germinating corn was almost stopped.

In conclusion, it can be said that catalase activity and respiration do not increase together in germinating corn as the temperature increases. At 20° C. a slight correlation between catalase activity and respiration seems apparent during the germination of the different strains of corn. But the same strains of corn show little or no correlation between catalase activity and respiration at 10° and 30° C.

These results may be interpreted as supporting the theory that catalase acts as a check on too rapid oxidation by the destruction of peroxids, but further work would be necessary to establish this theory. There is no evidence for the conclusion that catalase is the enzym most concerned in physiological oxidations.

Summary and Conclusions

1. The use of large quantities of peroxid to measure small amounts of catalase is objectionable, since an excess of peroxid retards the action of catalase.

2. The drying of corn seedlings at a temperature of 56° C. reduces their catalase content.

3. The drying of corn seedlings at room temperature *in vacuo* over sulfuric acid does not affect their catalase content.

4. The soaking of corn for one hour in a 0.25 percent solution of Uspulun effectively prevents the growth of molds. This treatment has no stimulating effect upon germination or catalase activity above that secured by soaking the corn for the same length of time in water.

5. The average catalase content of ungerminated corn measured in cubic centimeters of oxygen for a 10-minute period is as follows: High Protein, 13.5 cc.; High Oil, 11.6 cc.; Low Protein, 7.3 cc.; and Low Oil, 6.6 cc.

6. During the germination of these different strains of corn at all temperatures, catalase activity decreases in the early stages. This activity increases in the later stages of germination until it reaches a maximum, and this is then followed by a decrease as the reserve food of the grain is used.

7. The highest catalase activity during germination is found in the

High Oil strains; the High Protein strains giving next highest, the Low Protein strains the next, and the Low Oil strains the lowest.

8. There is a gradual accumulation of catalase in corn germinating at 10° C. so that ultimately the catalase content is equal or nearly equal to that found during germination at the higher temperatures.

9. Catalase activity in germinating corn at 30° C. does not increase proportionally to the increased metabolism caused by the higher temperature. In most cases, the catalase content at 30° is less than that at 20° C.

10. A temperature of 42° C. reduces markedly the catalase content of corn seedlings.

11. Respiration increases slowly in the early stages of germination and then more rapidly in the later stages, while catalase activity at first decreases and then increases.

12. Respiration increases rapidly with an increase in temperature, while catalase fails to show a corresponding increase.

13. Some correlation between catalase activity and respiration is apparent during the germination of the different strains of corn at 20° C., but at 10° and 30° C. this correlation is not apparent.

14. The results obtained fail to show a close correlation between catalase activity and respiration. There is no evidence for concluding that catalase is the enzym chiefly concerned in physiological oxidation. The evidence more nearly supports the theory that catalase prevents excessive oxidation.

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VITA

Cyrus William Lantz was born June 27, 1889, at Brooklyn, Illinois. He graduated from the High School at Brooklyn, Illinois, in 1906, after which he attended the Western Illinois State Normal School, Macomb, Illinois, graduating in 1909.

He entered the University of Illinois in 1911 and received the A.B. degree with final honors in 1913. The following year he received the A.M. degree. He was enrolled in the Graduate School of the University of Illinois 1913–1915, 1919, and 1924–1925. He was a scholar in Botany, 1914–1915, and fellow in Botany, 1919, in the University of Illinois.

He has held the following teaching positions: Principal of the High School, La Harpe, Illinois, 1909–1911; Assistant in Botany, University of Illinois, 1913–1914; Instructor in Biology, Thornton Township High School, Harvey, Illinois, 1915–1917; Assistant Professor of Botany and Horticulture, University of Nevada, 1917–1921; and Professor of Botany, Iowa State Teachers' College, 1921–.

He served nine months in the United States Army, 1918–1919, where he was engaged in a hookworm survey of the southern camps with the Sanitary Corps.

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